Osmolality Threshold for Erythrocyte Hemolysis

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ABSTRACT

In this study, we defined the minimum osmolality threshold for normal and sickle red cell hemolysis. Postmortem findings from water intoxication deaths are limited to edema of the brain and lungs with osmolality of 108 mEq/L (216 mOsm/kg), which is far lower than the physiological osmolality of 135–145 mEq/L (270–295 mOsm/kg). We investigated whether such low osmolality had any effect on the integrity of the erythrocyte membrane and to what extent. We hypothesized that red cell membrane's ability to deform/reform under shear confirms that the cell is highly resistant to changes in serum osmolality. Appropriately, collected whole blood was centrifuged to separate plasma from red cells. The packed cells were washed three times and resuspended (~25% hematocrit) in isotonic solution. A total of 50 µl of the 25% suspension was incubated in solution ranging from 290 to 65 mOsm/kg sodium chloride. Following incubation, the supernatant and pellets were analyzed for hemoglobin (spectrometry) and glycophorin A content by using Western blotting techniques. Red cells hemolyzed when osmolality dropped to less than 95 mEq/L (190 mOsm/kg) and 170 mOsm/kg for normal and sickle erythrocytes, respectively. Below 190 mOsm/kg and 170 mOsm/kg (sickle cell), membrane rupture was rapid, displaying an S-shaped "cooperativity" pattern similar to that of oxygen-hemoglobin–binding curve. Complete (100%) hemolysis occurred at ≤75 mEq/L (150 mOsm/kg). Hemoglobin content was ~50% lower in cells exposed to hypotonic compared with isotonic or hypertonic solutions. Erythrocytes comparatively show more resilience to changes in osmolality, remaining intact at 216 mOsm/kg because of their flexible membrane and cytoskeletal network of proteins. These findings provide insights into how normal patients, patients with sickle cell, and perhaps older patients would withstand changes in serum osmolality during dehydration/rehydration states. To alleviate their pain, intravenous fluids are routinely administered, irrespective of the hydration status to slow or reverse the sickling process. Hence, electrolyte balance and fluid volume replacements during acute episodes of pain may significantly benefit patients afflicted by the sickle cell disease.

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